IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

14/declaration 1.132

In re Patent Application of

Parikh et al

Atty. Ref.: 121-184

Serial No. 09/443,863

Group: 1615

Filed: November 19, 1999

Examiner: Kishore

For: DISPERSIBLE PHOSPHOLIPID STABILIZED MICROPARTICLES

* * * * * * * * * * *

Assistant Commissioner for Patents
Washington, DC 20231

Sir:

EVIDENTIARY DECLARATION UNDER 37 C.F.R. §1.132

I, Awadhesh Mishra, the inventor in the above-identified application, do hereby declare:

1. That my residence and citizenship are of record in this application as stated in my declaration as inventor made under 37 C.F.R.§1.63.

2. That I am familiar with the contents of the above-identified application and the research effort underlying this application, and that I have read and am familiar with the Official Action of August 21, 2001, cited in the Official Action.

3. That the following experiments were conducted by me or under my direction and control and that the results obtained and reported herein are accurate.

Comparison between a product of the process of Green et al. in U.S. 5,976,577: "Process for preparing fast dispersing solid oral dosage form," ('577) with a product of the process of the above-identified patnet application, Parikh et al. '863.

Method: Compare particle size distributions and microscopy observations of samples before lyophilization and after rehydration of particles of fenofibrate prepared

according to Green et al. ('577), claim 1, having an initial size range from 50 μm to 400 μm versus microparticles of Parikh et al. ('863).

Materials used are reported with comments in Table 1, below.

Table 1. Materials used to prepare particles according to the method of Green et al. in U.S. 5976577

Batch size = 100 grams

Ingredient	Composition (%w/w)
Lipoid E-80 (a phospholipid, see Green et al, col. 5, line 47)	3.0
Fenofibrate, a lipid lowering drug (see Green et al., col. 8, line 29) sieved in order to obtain a 50 to 400 μm particle size distribution (see Green et al., col. 5, lines 7-8 and claim 10)	10.0 (see Green col. 5, line 30)
Sorbitol, a polyhydric structure-forming agent (see Green et al., col. 6, lines 36-38 and claims 11 and 12)	5.0
Sucrose (see Green et al., col. 6, lines 36-37 and claim 11)	15.0
Water as phosphate buffer 10 mM pH = 8 (see Green et al., col. 5, line 54)	Qs to 100%
Sodium hydroxide	For pH adjustment
Hydrochloric Acid	For pH adjustment

(a) Preparation of large particles using the hot melt encapsulation method of Green et al. (col. 5, line 24)

A non-aqueous phase composed of phospholipid (Lipoid E-80) and solid drug (fenofibrate), and a water phase composed of the structure-forming agents sucrose and

sorbitol (see Green et al., column 6, lines 36-38) in an aqueous phosphate buffer solution were both heated to 80-90°C under N_2. The heated non-aqueous phase was then transferred to the heated water phase. The mixture was sonicated for 40 minutes at 85-90°C to obtain a fluid suspension of fenofibrate particles coated with Lipoid E-80. An aliquot was removed for particle size distribution measurement and microscopic observations. The remaining heated suspension was poured into molds in blister trays, frozen, and lyophilized according to the method of Green et al. (see col. 6, lines 53 to col. 7, line 60).

After lyophilization, the remaining material in the individual molds was reconstituted from its powder form with deionized water in a 1:1 ratio. The particle size distribution of the resulting resuspended material was measured and the suspension was observed under an optical microscope.

(b) Preparation of a dosage form of fenofibrate according to the present invention

A premix containing a phospholipid (Lipoid® E80, 120.3 g) and a solid drug (fenofibrate, 400.9 g) in pharmaceutical grade water (3480 g) was well mixed to a visually homogeneous suspension using a rotor/stator type (Quadro Y0) mixer with occasional assistance with a plastic spatula. The pH of premix was 6.7. Optical microscopic examination of an undiluted sample of this premix suspension using an Olympus BH2 microscope in bright field with 125X magnification revealed the presence of large, relatively free-flowing crystals and some lipid vesicles before microfluidization.

This premix was poured into the inlet reservoir of a high pressure microfluidizer equipped with a diamond interaction chamber (Microfluidics model M210B, MFIC Corporation, Newton, MA), and recirculated at low pressure (3000 psig) through the

processing fluid loop of the equipment to achieve thermal equilibration at an initial temperature range of 1-7°C in the inlet reservoir. The process pressure was increased slightly as necessary to prevent clogging. The premix under circulation through the high pressure homogenization device is referred to as the processing fluid. The premix was then microfluidized (i.e., homogenized) at 18,000 psig in recirculating mode until the resulting homogeneous suspension exhibited a volume weighted average particle size of less than 1.00 μm measured using a Malvern Mastersizer.

The product was harvested into a collection vessel at the minimum operating pressure of the microfluidizer. Gross yield of this process was 83.2%, giving 3328.3 g of a homogeneous white suspension product with a pH of 6.71 at 12.5°C. Optical microscopy with an Olympus BH2 microscope in bright field with 1250X magnification of a 1:1 water diluted sample of the product suspension revealed presence of very small irregular "potato" shaped, free-flowing, particulate entities and no lipid vesicles. When measured with a Malvern Mastersizer Microplus particle sizer, the suspension displayed a particle size distribution with a volume weighted mean diameter of 0.98 μm with the 50 and 100 percentiles of population being 0.92 and 2.28, respectively. The distribution was monomodal with Mode 1 measuring at 0.95 μm.

An aliquot of the homogenized suspension was then transferred at ambient temperature to a 100 mL glass bottle containing the bulking/releasing agents sorbitol (at 5% w/w) and sucrose (at 15 % w/w) as dry powders. This mixture was magnetically stirred to rapidly dissolve the bulking/releasing agents.

Two milliliter aliquots of this mixture were then transferred into 10 mL glass vials which were then placed on the shelves of a Virtis lyophilizer. Lyophilization of the homogenized suspension containing the bulking agents was performed using the following cycle: (a) thermal equilibration at 5°C for 1 hour; (b) freeze to a shelf temperature of -50°C in 1 hr 50 min at approximately 0.5°C/min and equilibrate at -50°C for 1/2 hour; (c) apply vacuum pump; (d) hold at -50°C for 1/2 hour (e) set the shelf temperature to -25°C; (d) hold at -25°C for 60 hours; (f) set the shelf temperature to 20°C; (g) hold at 20°C until ready to release vacuum. At the end of this cycle the lyophilization chamber was purged with nitrogen gas, and the vials were stoppered. The resulting lyophilized cakes were homogeneous, uniform, smooth, crack-free, and shrinkage-free in the vials consistent with the absence of settling prior to freezing.

Five mL of simulated gastric fluid (SGF) (2g/L NaCl + 5mL/L HCl) was added to a sample vial which was gently inverted a few times to facilitate reconstitution of the cake. When measured with a Malvern Mastersizer Microplus particle sizer, the reconstituted suspension displayed a particle size distribution of a volume weighted mean diameter of 0.98 μm, and the 50 and 100 percentiles of population were 0.92 and 2.28, respectively. The distribution was monomodal with Mode 1 measuring at 0.95 μm. Optical microscopy of the reconstituted product using an Olympus BH2 microscope in bright field revealed the presence of a very fine dispersion of small, free-flowing, particles. No crystal growth or lipid vesicles were observed.

One of the lyophilized cakes in the stoppered vial was kept at 25°C and another at 60% relative humidity (RH) for 1 month, after which period the cake in each was

reconstituted with five mL each of SGF by gently agitation. When measured with a Malvern Mastersizer Microplus particle sizer, the reconstituted suspension displayed a particle size distribution of a volume weighted mean diameter of 0.96 μm, with the 50 and 100 percentiles of population at 0.92 and 2.28, respectively. The distribution was monomodal with Mode 1 measuring at 0.96 μm. Similarly, a lyophilized cake in the stoppered vial was kept at 40°C and 75% relative humidity (RH) for 12 days, after which period the cake was reconstituted with five mL of SGF by gently agitation. When measured with a Malvern Mastersizer Microplus particle sizer, the reconstituted suspension displayed a particle size distribution of a volume weighted mean diameter of 0.97 μm, and the 50 and 100 percentiles of population were 0.91 and 2.28, respectively. The distribution was monomodal with Mode 1 measuring at 0.95 μm.

RESULTS

1. Particle size distributions

The results obtained in the particle size analysis related to the product prepared according to Green et al. before lyophilization of the suspension and after reconstitution are shown in Figures 1 and 2 and in Table 2, below. The mean diameter of the Green et al. fenofibrate particles increased substantially after the lyophilization and reconstitution step. The mean diameter of the suspension before lyophilization is 3.88 μm but it increases to 29.39 μm after freeze-drying and rehydration. The graphical readouts of Figures 1 and 2 demonstrate substantial change to a larger size range in Figure 2 indicating the presence of agglomerates or aggregates having sizes of several hundred

microns. This result indicates that the particles do not redisperse to their original distribution after lyophilization and rehydration. However, as seen in Figures 3 and 4, the primary particles of the process of the current invention of Parikh et al. were reobtained with practically the same particle size dispersion after lyophilization and resuspension. This is unlike the coarse particles of Green et al. In Parikh et al. the smaller fenofibrate microparticles give a result unexpected in view of the results of Green et al. Further, there is no evidence of increase in particle size that might be expected by an Ostwald ripening process in the case of Parikh et al.

The Examiner will note that the particle size data are essentially those of Formulation number 6 in Table 1 of the current application of Parikh et al.

1A. Description of the Malvern Mastersizer Particle Size Distribution Figures 1 to 4.

Figure 1 is a copy of results of particle size distribution measurements obtained using a Malvern Mastersizer of a suspension of particles prepared by the method of Green et al. before lyophilization.

Figure 2 is a copy of results of particle size distribution measurements obtained using a Malvern Mastersizer of a suspension of particles prepared by the method of Green et al. after lyophilization and rehydration.

Figure 3 is a copy of results of microparticle size distribution measurements obtained using a Malvern Mastersizer of a suspension of particles prepared by the method of Parikh et al. of the current invention before lyophilization.

Figure 4 is a copy of results of microparticle size distribution measurements obtained using a Malvern Mastersizer of a suspension of particles prepared by the method of Parikh et al. after lyophilization and rehydration. (Note that the scale ranges in Figures 3 and 4 are slightly different, but this does not alter size distributions).

1B. The data in Table 2 are taken from Figures 1 through 4.

Table 2. Particle size distribution data from particles prepared by the method of Green et al. US

Parameter	**Figure 1 Green et al. Before lyophilization**	**Figure 2 Green et al.** After lyophilization	**Figure 3 Parikh et al. Before lyophilization**	**Figure 4 Parikh et al.** After lyophilization
Mean (μm)	3.88	29.39	0.98	0.97
Percentile	**Distribution Percentiles (μm)**			
10.0 %	0.61	6.86	0.58	0.59
20.0 %	0.81	12.17	0.67	0.68
50.0 %	1.70	24.53	0.92	0.91
80.0 %	5.69	42.16		1.22
90.0 %	9.85	54.56	1.48	1.43
95.0 %			1.69	
99.0 %	27.82	118.93	2.03	1.98
99.9 %	40.21	246.38	2.21	2.19
100.0 %	48.27	301.68	2.28	2.28

2. Optical microscopy observations

Optical micrographs of the particles of Green et al., are presented below as Figures 5A (125X magnification) and 5B (1250X magnification), and Figures 6A and 6B. These optical micrographs confirm the results obtained in the particle size analysis of Figures 1 and 2. It can be observed that the fenofibrate particles of Green et al. in the initial suspension before lyophilization are dispersed and not agglomerated (Figures 5A-5B).

However, after lyophilization and rehydration, the particles of Green et al. tend to agglomerate and not fully dissociate. The rehydrated particles of Green et al. thus do not give a suspension that is similar to the suspension before lyophilization. The presence of agglomerates (Figures 6A-6B) results in an increase in the measured particle size distribution. This is consistent with the particle size data in Table 2 and in Figure 2 relative to Figure 1.

When viewed under an optical microscope, a suspension of the microparticles of the process of Parikh before lyophilization (see Figures 7 and 8) was essentially without aggregates and was the same as a suspension formed after lyophilization and rehydration (see Figure 9 and 10). This is consistent with the particle size distribution measurements of Figures 3 and 4.

Optical microscopy of the prelyophilized suspension as well as of lyophilized and rehydrated product with an Olympus BH2 binocular microscope in dark field revealed the presence of a very fine dispersion of small, free-flowing, crystalline particles. Overall magnification of the microscope was 500X at the binocular eye piece. No crystal growth or lipid vesicles were observed. No agglomerates or flocculates were observed. Single particles were seen rapidly moving in a Brownian motion pattern although some of the particles were more mobile than others.

Magnified images of the view-fields were captured photographically with an Olympus OM-2S camera (format: 35 mm) equipped with a black and white negative film (speed: 3200 ASA) attached to the microscope. The camera was operated with fully open aperture in auto mode that adjusted the shutter speed automatically to allow a balanced exposure. The negative film was processed to obtain positive photographic plates with

further enhancement of magnification. The photographs were scanned with a Hewlett Packard ScanJet 4c scanner into electronic image files from which they are reproduced below. Figures 7 and 8 show the suspension prior to lyophilization and Figures 9 and 10 show reconstituted suspension after lyophilization. Some of the particles in the image appear blurred due to Brownian motion or due to particles positioned out of the focal plane of the camera. Linear motion of some of the particles was captured in one of the photographs due to long exposure time. Overall, presence of single unagglomerated or unflocculated particles was confirmed in the samples of the original suspension as well as lyophilized and rehydrated dosage form suspension of the current invention.

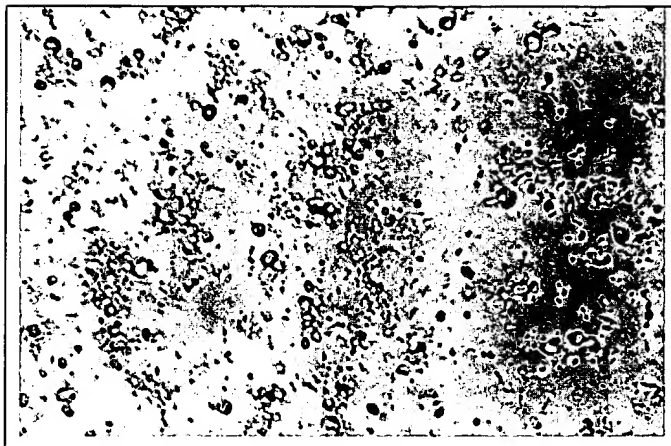


Figure 5A

Suspension of Green et al. before lyophilization X125

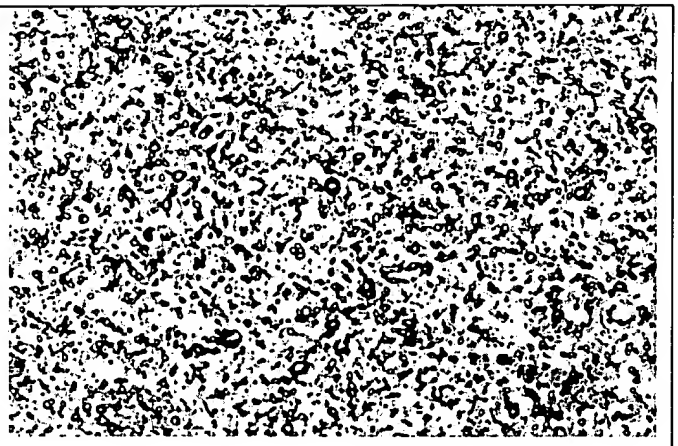


Figure 5B

Suspension of Green et al. before lyophilization X1250

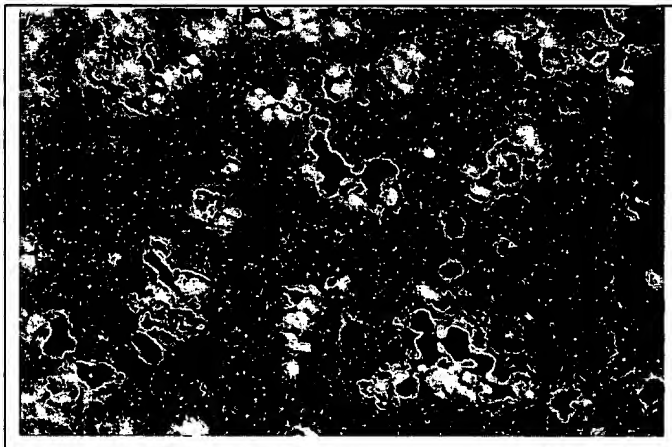


Figure 6A

Suspension of Green et al. after lyophilization and rehydration; X125

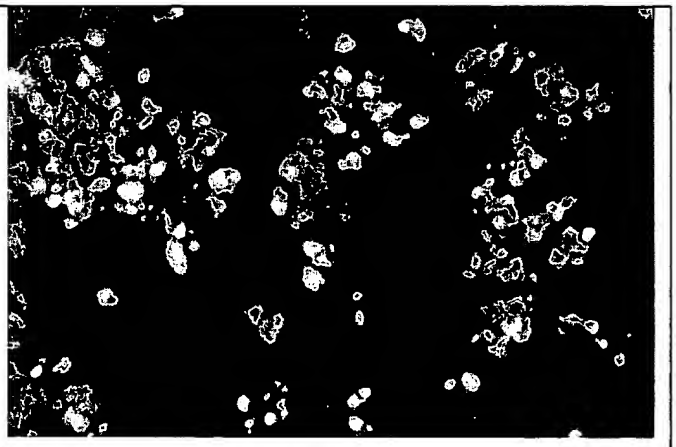


Figure 6B

Suspension of Green et al. after lyophilization and rehydration; X500

Figure 7: Phospholipid stabilized fenofibrate microparticle suspension prepared according to the current invention before lyophilization. Track due to free flow is visible for some of the particles.

Figure 8: Phospholipid stabilized fenofibrate microparticle suspension prepared according to the current invention prior to lyophilization, (2nd picture)

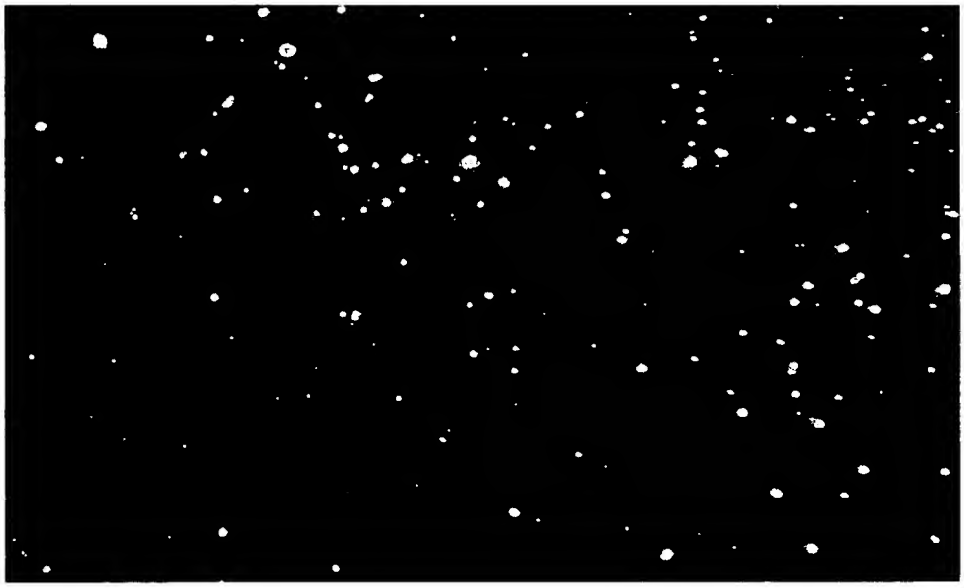
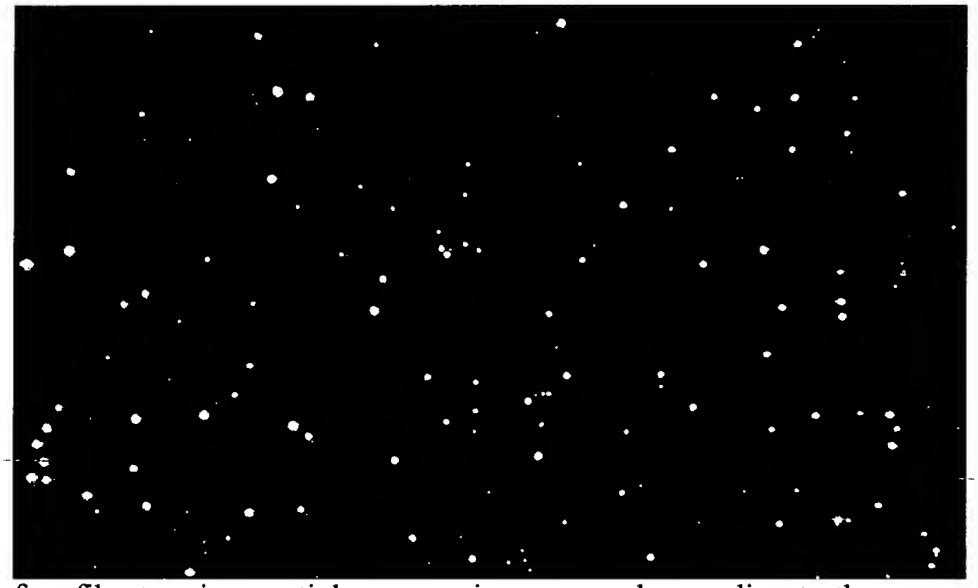
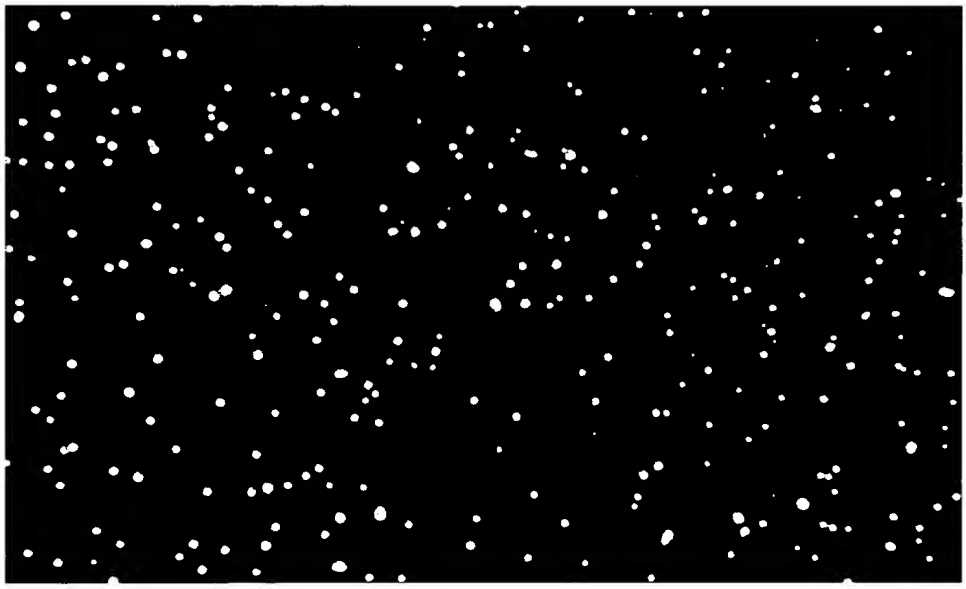


Figure 9: Rehydrated suspension after lyophilization of phospholipid stabilized



fenofibrate microparticle suspension prepared according to the current invention.

Figure 10: Rehydrated suspension after lyophilization of phospholipid stabilized fenofibrate microparticle suspension prepared according to the current invention (2nd picture).



I declare further that all statements made herein of my/our own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

Date: August 08, 2001

Awadhesh Mishra

Figure 1 Particle size distribution of coated coarse particles prepared according to Green et al. before lyophilization

MALVERN INSTRUMENTS MASTERSIZER

Result Derived Diameters Report

Sample Details

Sample ID: IDD-P-Fenofibrate
Sample File: 010215PC
Sample Path: M:\CHEM_0~1\2001\02_FEB~1\
Sample Notes: Before lyophilization
90 degrees process

PC

Run Number: 2
Record Number: 2

Measured: Feb 15 2001 2:48PM
Analysed: Feb 15 2001 2:48PM
Result Source: Analysed

System Details

Sampler: Internal
Presentation: 5RHD
Analysis Model: Polydisperse
Modifications: None

[Particle R.I. = (1.9285, 0.1000); Dispersant R.I. = 1.3300]

Measured Beam Obscuration: 13.2 %
Residual: 0.681 %

Result Statistics

Distribution Type: Volume
Concentration = 0.0023 %Vol
Span = 5.424E+00
Density = 1.000 g / cub. cm
Uniformity = 1.718E+00
Specific S.A. = 4.4731 sq. m / g

A.S.T.M Derived Diameters (um)

N	3	2	1	0
D[4, N]	3.88	2.28	1.60	1.25
D[3, N]		1.34	1.03	0.86
D[2, N]			0.78	0.69
D[1, N]				0.60

Distribution Moments

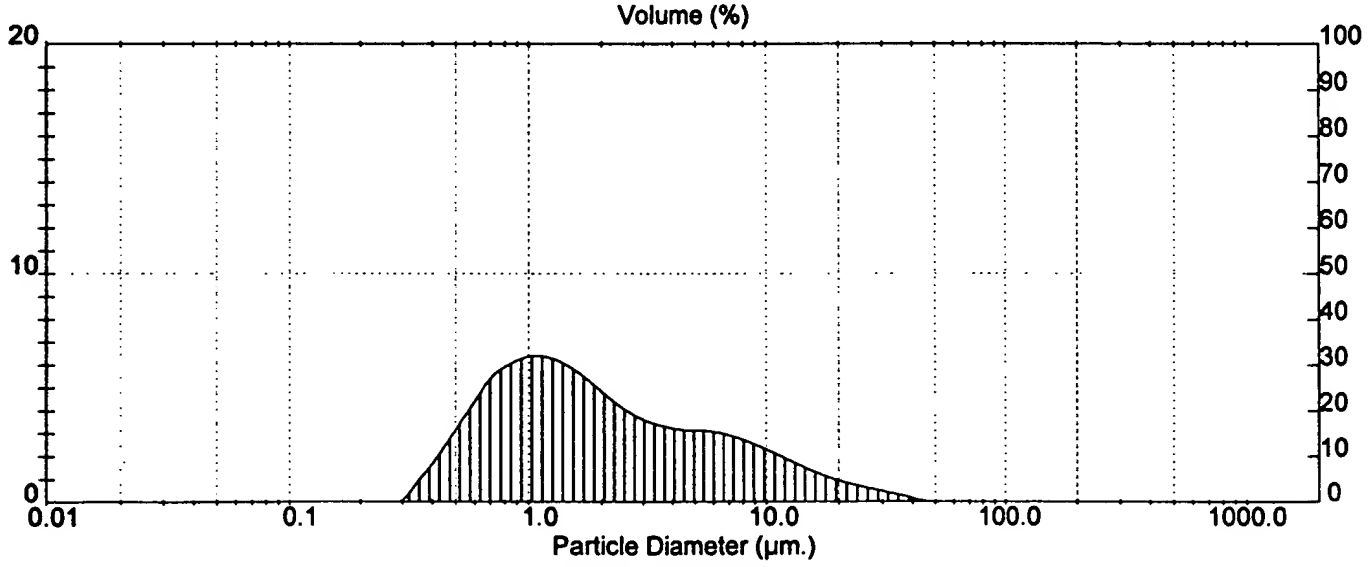
	Mean	Stan. Dev.	Skewness	Kurtosis
Volume	3.88	5.407	3.0923	12.0381
Surface	1.34	1.846	6.8833	76.0878
Length	0.78	0.661	8.9025	188.2346
Number	0.60	0.331	6.0113	128.0114

Distribution Percentiles (um) – Volume

Percentile	Size	Percentile	Size
10.0 %	0.61	90.0 %	9.85
20.0 %	0.81	99.0 %	27.82
50.0 %	1.70	99.9 %	40.21
80.0 %	5.69	100.0 %	48.27

Distribution Modal Sizes (um)

Mode	Size	Mode	Size
1	1.07	2	5.10



Malvern Instruments Ltd.
Malvern, UK
Tel:+[44] (0)1684-892456 Fax:+[44] (0)1684-892789

Mastersizer Microplus Ver. 2.18
Serial Number: 33370-47

15 Feb 01 14:4

Figure 2 Particle size distribution of coated coarse particles prepared according to Green et al. after lyophilization and rehydration

MALVERN INSTRUMENTS MASTERSIZER

Result Derived Diameters Report

Sample Details

Sample ID: IDD-P-Fenofibrate
Sample File: 010215PC
Sample Path: M:\CHEM_0~1\2001\02_FEB~1\
Sample Notes: After lyophilization, reconstitution with water
90 degrees process

PC

Run Number: 1
Record Number: 3

Measured: Feb 15 2001 3:12PM
Analysed: Feb 15 2001 3:12PM
Result Source: Analysed

System Details

Sampler: Internal
Presentation: 5RHD
Analysis Model: Polydisperse
Modifications: None

[Particle R.I. = (1.9285, 0.1000); Dispersant R.I. = 1.3300]

Measured Beam Obscuration: 13.3 %
Residual: 0.251 %

Result Statistics

Distribution Type: Volume
Concentration = 0.0201 %Vol
Span = 1.945E+00
Density = 1.000 g / cub. cm
Uniformity = 6.436E-01
Specific S.A. = 0.7789 sq. m / g

A.S.T.M Derived Diameters (um)

N	3	2	1	0
D[4, N]	29.39	15.05	4.47	1.89
D[3, N]		7.70	1.74	0.76
D[2, N]			0.39	0.24
D[1, N]				0.14

Distribution Moments

	Mean	Stan. Dev.	Skewness	Kurtosis
Volume	29.39	24.914	3.4761	22.2577
Surface	7.70	12.926	3.2960	23.8285
Length	0.39	1.695	17.5631	524.7657
Number	0.14	0.189	61.2385	9749.4775

Distribution Percentiles (um) -- Volume

Percentile	Size	Percentile	Size
10.0 %	6.86	90.0 %	54.56
20.0 %	12.17	99.0 %	118.93
50.0 %	24.53	99.9 %	246.38
80.0 %	42.16	100.0 %	301.68

Distribution Modal Sizes (um)

Mode	Size	Mode	Size
1	31.68		

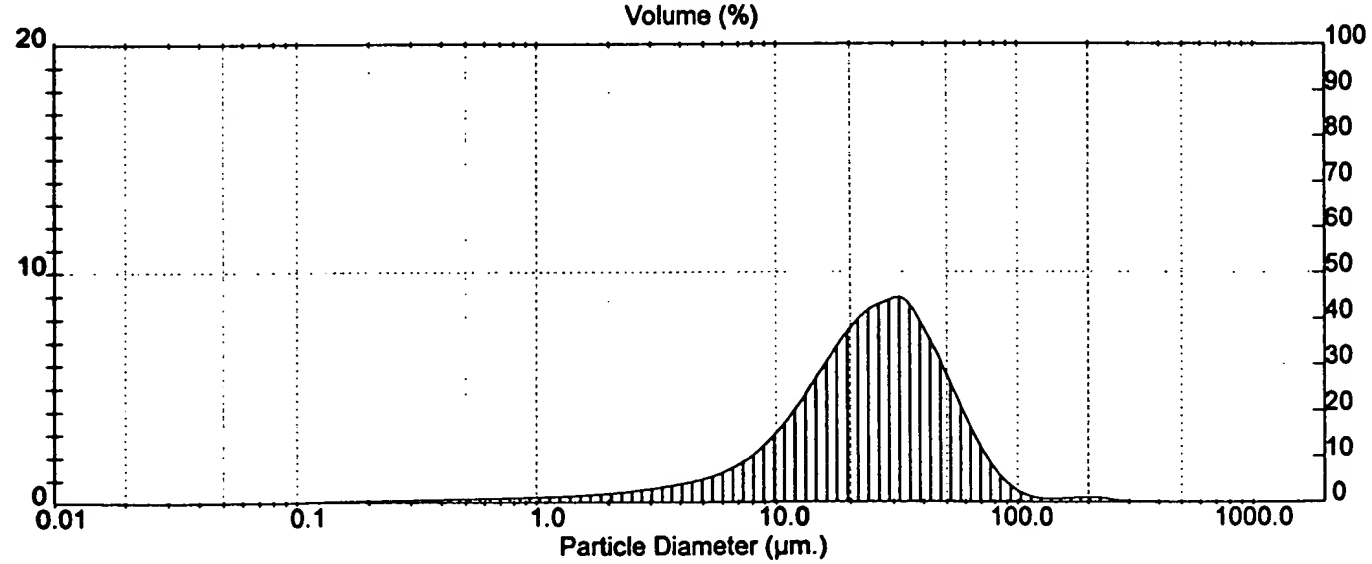


Figure 3. rticle size distribution of micropart[illegible]s prepared according the present invention of Parikh et a[illegible]efore lyophilization

MALVERN INSTRUMENTS MASTERSIZER

Result Derived Diameters Report

Sample Details

Sample ID: IDD-P Fenofibrate
Sample File: 991004
Sample Path: C:\SIZERMP\DATA\1999\SEPTEMBE\
Sample Notes: M2108
991004.45.144
Final Harvested Suspension

Run Number: 8
Record Number: 20

Measured: Mon Oct 4 1999 5:44PM
Analysed: Mon Oct 4 1999 5:44PM
Result Source: Analysed

System Details

Sampler: Internal
Presentation: 5RHD
Analysis Model: Polydisperse
Modifications: None

[Particle R.I. = (1.9285, 0.1000); Dispersant R.I. = 1.3300]

Measured Beam Obscuration: 11.5 %
Residual: 0.800 %

Result Statistics

Distribution Type: Volume
Concentration = 0.0012 %Vol
Span = 9.890E-01
Density = 1.000 g / cub. cm
Uniformity = 3.060E-01
Specific S.A. = 6.9586 sq. m / g

A.S.T.M Derived Diameters (um)

N	3	2	1	0
D[4, N]	0.98	0.92	0.86	0.81
D[3, N]		0.86	0.81	0.77
D[2, N]			0.76	0.72
D[1, N]				0.68

Distribution Moments

	Mean	Stan. Dev.	Skewness	Kurtosis
Volume	0.98	0.359	0.8910	0.5569
Surface	0.86	0.318	1.1123	1.4186
Length	0.76	0.275	1.2945	2.3188
Number	0.68	0.235	1.4374	3.1437

Distribution Percentiles (um) – Volume

Percentile	Size	Percentile	Size
10.0 %	0.58	95.0 %	1.69
20.0 %	0.67	99.0 %	2.03
50.0 %	0.92	99.9 %	2.21
90.0 %	1.48	100.0 %	2.28

Distribution Modal Sizes (um)

Mode	Size	Mode	Size
1	0.95		

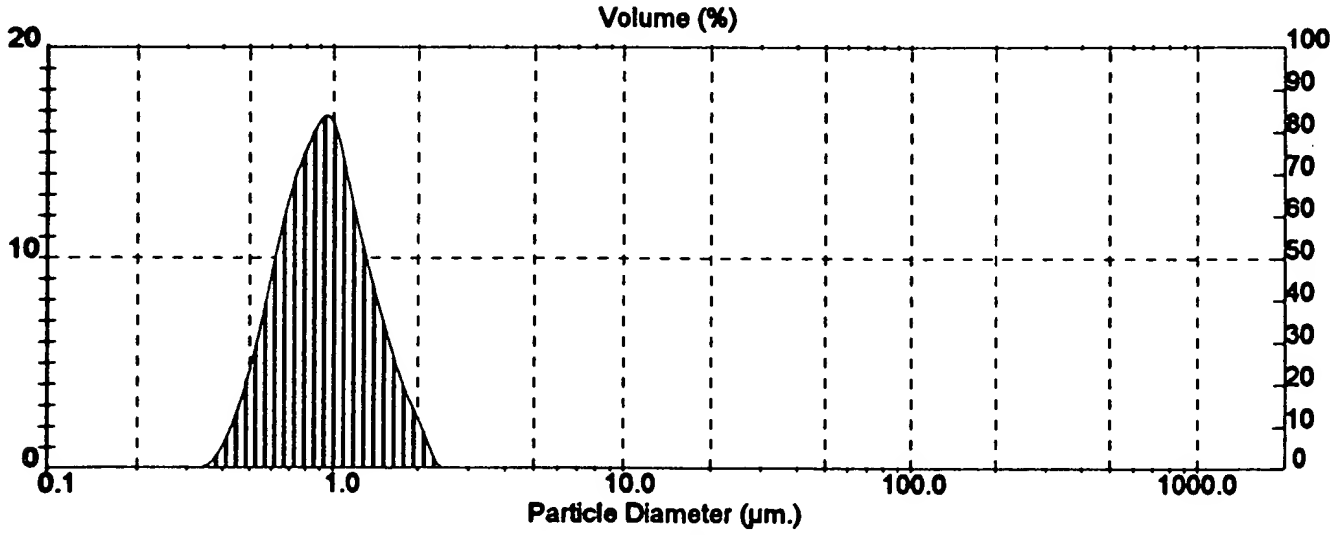


Figure 4. Particle size distribution of micropa[illegible]les prepared according [illegible]o the present invention of Parikh et [illegible] after lyophilization and rehydration

MALVERN INSTRUMENTS MASTERSIZER

Result Derived Diameters Report

Sample Details

Sample ID: IDD-P Feno
Sample File: 991115
Sample Path: C:\SIZERMP\DATA\1999\NOVEMBER\
Sample Notes: IDD-P Fenofibrate Batch# 991004.45.144
Lyo cycle 91004.02.145, stored for 1mth @ ambient conds.
+ 5%w/w sorbitol/15%w/w Sucrose, stored at 40C/75%RH for
12 dys, seal. Recon with 5ml mod SGF, 30 inv. (SGF), + pip.

Run Number: 17
Record Number: 18

Measured: Mon Nov 15 1999 3:54PM
Analysed: Mon Nov 15 1999 3:55PM
Result Source: Analysed

System Details

Sampler: Internal
Presentation: 5RHD
Analysis Model: Polydisperse
Modifications: None

[Particle R.I. = (1.9265, 0.1000); Dispersant R.I. = 1.3300]

Measured Beam Obscuration: 11.6 %
Residual: 0.868 %

Result Statistics

Distribution Type: Volume
Concentration = 0.0012 %Vol
Span = 9.206E-01
Density = 1.000 g / cub. cm
Uniformity = 2.871E-01
Specific S.A. = 6.9293 sq. m / g

A.S.T.M Derived Diameters (um)

N	3	2	1	0
D[4, N]	0.97	0.92	0.87	0.82
D[3, N]		0.87	0.82	0.78
D[2, N]			0.78	0.74
D[1, N]				0.70

Distribution Moments

	Mean	Stan. Dev.	Skewness	Kurtosis
Volume	0.97	0.338	0.9228	0.7699
Surface	0.87	0.301	1.0951	1.5036
Length	0.78	0.264	1.2295	2.1933
Number	0.70	0.229	1.3337	2.7758

Distribution Percentiles (um) -- Volume

Percentile	Size	Percentile	Size
10.0 %	0.59	90.0 %	1.43
20.0 %	0.68	99.0 %	1.96
50.0 %	0.91	99.9 %	2.19
80.0 %	1.22	100.0 %	2.28

Distribution Model Sizes (um)

Mode	Size	Mode	Size
1	0.95